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(54) ENZYMES AYANT UNE ACTIVITE DE LIPASE/ACYLTRANSFERASE
(54) ENZYMES WITH LIPASE/ACYLTRANSFERASE ACTIVITY

(57)

Polypeptides with lipase/acyltransferase activity are proposed with an amino acid sequence which has identity to the sequence reported in SEQ ID NO 2 of at least 49%, also polypeptides which display this activity, further nucleic acids (genes) which code for these polypeptides, vectors which contain nucleic acids which code for these polypeptides, transformed microorganisms which contain these nucleic acids, processes for production of these polypeptides and the application of nucleic acids for discovering new lipase/acyltransferases and the use of these lipase/acyltransferases as catalysts in chemical and biochemical processes.

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(54) Titre : ENZYMES AYANT UNE ACTIVITE DE LIPASE/ACYLTRANSFERASE
(54) Title: ENZYMES WITH LIPASE/ACYLTRANSFERASE ACTIVITY

(57) Abrégé/Abstract:
Polypeptides with lipase/acyltransferase activity are proposed with an amino acid sequence which has identity to the sequence reported in SEQ ID NO 2 of at least 49%, also polypeptides which display this activity, further nucleic acids (genes) which code for these polypeptides, vectors which contain nucleic acids which code for these polypeptides, transformed microorganisms which contain these nucleic acids, processes for production of these polypeptides and the application of nucleic acids for discovering new lipase/acyltransferases and the use of these lipase/acyltransferases as catalysts in chemical and biochemical processes.

ABSTRACT

Polypeptides with lipase/acyltransferase activity are proposed with an amino acid sequence which has identity to the sequence reported in SEQ ID NO 2 of at least 49%, also polypeptides which display this activity, further nucleic acids (genes) which code for these polypeptides, vectors which contain nucleic acids which code for these polypeptides, transformed microorganisms which contain these nucleic acids, processes for production of these polypeptides and the application of nucleic acids for discovering new lipase/acyltransferases and the use of these lipase/acyltransferases as catalysts in chemical and biochemical processes.

TITLE OF THE INVENTION

Enzymes With Lipase/Acyltransferase Activity

BACKGROUND OF THE INVENTION

The present invention concerns polypeptides with lipase/acyltransferase activity, amino acids sequences of polypeptides, which display this activity, nucleic acids (genes) which code for these polypeptides, vectors containing the nucleic acids which code for these polypeptides, transformed microorganisms which contain these nucleic acids, a process for the production of polypeptides and the application of nucleic acids for discovering new lipase/acyltransferases and the use of the lipase/acyltransferases as catalysts in chemical and biochemical processes.

During esterification by conventional methods of chemical synthesis, on the one hand, because of the presence of several free hydroxyl groups in the alcohol component or one of their partial esters, usually mixtures of mono- and polysubstituted products are formed so that the introduction and removal of protective groups is necessary if it is desired to synthesize a certain compound selectively.

Through the use of activated carboxylic acid derivatives secondary products are formed and frequently also undesired secondary products which makes refining difficult, reduces the yields of desired product and pollutes the environment. These disadvantages can be avoided or at least reduced by conducting the process by the enzymatic route.

In chemical and biochemical synthesis enzymes are increasingly used as catalysts. Thus in many cases because of the frequently milder reaction conditions hydrolases, especially lipases are already used for splitting fats in large scale industrial processes.

Enzymatic processes for the esterification or reesterification are well known in the art.

It is also known that transesterification can be catalyzed by lipases in water-free media. If water is present in the reaction system of esters, alcohol and lipases, cleavage of the acids normally occurs. Since various lipases also catalyze the formation of esters from free fatty acids and alcohols, lipase catalyzed transesterifications normally proceed through an acid intermediate stage. However, in many commercial processes, the presence of free acids is undesirable. The water content

prevents to some extent a technically and commercially acceptable reaction (formation of an unfavorable thermodynamic equilibrium). Thus, costly operations such as water removal by such methods as azeotropic distillation, membrane separation processes, vacuum distillation have to be employed in order to achieve satisfactory yields.

A unique polypeptide has been described which displays lipase/acyltransferase activity. This polypeptide was isolated from the microorganism *Candida parapsilosis* CBS 604. To date, however, only the enzymatic activity of this polypeptide has been evaluated. In transesterification reactions catalyzed by the enzyme from *Candida parapsilosis* it is possible to maintain a free acid concentration even in the presence of water (with an activity > 0.8) thereby avoiding costly water removal steps.

The disadvantage of enzymatically catalyzed reactions is frequently the availability and stability of the polypeptides.

Description of the invention

An object of the present invention to characterize and make available polypeptides which make acyl transfer possible in high yields even in an aqueous medium, thereby overcoming the conventional disadvantages of lipase-catalyzed esterification.

Another object of the present invention is the isolation and sequence determination of the amino acid and nucleotides of the polypeptides.

Another object of the present invention is the over production of the lipase/acyltransferase Also included in this object are transformed host cells which are capable of producing the polypeptides.

The term "polypeptide" refers to a polymer composed of natural amino acids. In the present application the 19 proteinogenic naturally occurring L-amino acids are designated by the internationally conventional 1 and 3 letter codes. Another designation is also protein, in which case the number of monomer units in the polypeptide should be limited to at least 50.

The term "lipase/acyltransferase activity" refers to the activity of a polypeptide or enzyme which combines the properties of lipases with the properties of acyltransferase. Lipases belong to the group of hydrolases (especially the esterases) which split specific fats (triglycerides) into glycerin and fatty acids; this process, called lipolysis, takes place at the phase boundary between fat and water. An important property leading to classification into the group of hydrolases is the surface activity of lipases. Mechanistically in the catalysis a catalytic triad of serine, histidine and aspartic acid (or glutamic acid) plays a part. Acyltransferases are also designated as transacylases and belong to the group of transferases. Quite generally they transfer acyl or especially acetyl groups from a donor to an acceptor molecule and are therefore of particular importance in the buildup and breakdown of fats. Studies on the lipase/acyltransferase according to the invention have shown that this polypeptide is surface active and catalyzes reaction which are characteristic for lipases. It has also been found that this polypeptide is capable of catalyzing transesterifications at a water content in the reaction mixture corresponding to a water activity greater than 0.8. At this content of water a conventional lipase would catalyze only the hydrolysis of esters. Therefore, the polypeptides according to the invention exhibit both the characteristic features of lipases and also of acyltransferases. In the case of the naturally occurring polypeptide according to the invention, based on the sequence homologies with previously well known enzymes such as lipases from *Candida albicans* this involves a lipase, and on the basis of its enzymatic activity, it involves an acyltransferase.

As preferred donors in the sense of the invention for catalytic reactions with the lipase/acyltransferase according to the invention all possible esters, fats, triglycerides, 1,3-diglycerides, 1,2-diglycerides and 1-monoglycerides are used. As preferred acceptors in the sense of the invention for catalytic reactions with the lipase/acyltransferase according to the invention primary and secondary alcohols with 1 to 5 carbon atoms, especially ethanol, propanol, butanol, 1,2-propane diol, 1,3-propane diol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and hydroxylamines may be used.

The term "identity" used here with respect to the amino acid sequence designates a homology to the given amino acid sequence which leads to the fact that the polypeptide with a stated identity possesses the same biological activity as the first polypeptide. The identity of the nucleotide sequence pertains to a gene homologous to a first nucleotide sequence. Homologous with respect to the nucleotide sequence means that the gene may be allelic. Homologous also means that the gene may stem from another species and that the polypeptide coded by this gene has the same biological activity as the polypeptide coded by the first nucleotide sequence.

The subject of the invention is polypeptides with lipase/acyltransferase activity with an amino acid sequence which possesses identity to the amino acid sequence reported in SEQ ID NO.2 of at least 49%, preferably 80%, preferably at least 98%, especially preferably 99.8% and in particular 100%. An identity to the amino acid sequence reported in SEQ ID NO.2 of at least 96% is true especially for the partial region which corresponds to the amino acids in the positions 190 to 390. A polypeptide which possesses 100% identity to the amino acids sequence reported in SEQ ID NO.2 contains 465 amino acids. A 96% identity is especially preferred for the partial regions in positions 190-200, 220-290 and 330-385, especially for positions 196, 240 and 381.

The amino acid sequence of the enzyme according to the invention is reported in the sequence protocol under the designation SEQ ID NO.2. The nucleotide sequence of this enzyme is reported in the sequence protocol under the designation SEQ ID NO.1. It is therefore available for further developments via well known molecular biological methods.

Comparable polypeptides with lipase/acyltransferase activity also represent preferred variants of the present invention and are claimed to the extent that they display amino acid and/or nucleic acid sequences which lie within the range of similarity to the sequences reported in SEQ ID NO.1 and/or SEQ ID NO.2. This similarity range includes all polypeptides whose amino acid sequence is identical to 49%, to 80% to 96%, to 96.5%, to 97%, to 97.5% to 98%, to 98.5% to 99% to 99.5%, to 99.8% or to 100% to the amino acids sequence reported in SEQ ID NO2. This is especially true for those partial regions of the protein which involve amino acids 190-390.

A polypeptide which possesses identity of at least 80% to the amino acid sequence reported in SEQ ID NO 2 has a molecular weight between 49 and 55 kD after deglycosylation, especially 54 kD. The pH optimum for the catalytic reaction of transesterification, hydrolysis or esterification which was determined at 28°C is between 3 and 8.5, preferably between 4 and 8, especially between 6 and 7.5.

An optimal temperature range during the catalysis of the hydrolysis determined at the pH optimum is between 30 and 50°C, preferably between 35 and 40°C. An optimal temperature range for the catalysis of transesterification and esterification determined at the pH optimum is between 20-50°C, preferably between 20 and 30°C.

The polypeptides according to the invention also include those enzymes which display sufficient similarity to them or can be derived by known methods.

In a special variant of the inventions the polypeptides with lipase/acyltransferase activity are present glycosylated with an amino acid sequence which posess identity to the amino acid sequence reported in SEQ ID NO. 2 of at least 49%, preferably 80%, preferably at least 98%, especially preferably 99.8% and in particular 100%. The positions on which the polypeptide is present glycosylated and the degree of glycosylation depend on the organism producing this polypeptide. A degree of glycosylation of 1 to 2 sugar radicals per molecule of polypeptide is preferred.

In another variant of the invention the polypeptides are linked to another peptide. This peptide may involve a marker which, for example, may cause the desired polypeptide to be purified more effectively in chromatography, especially affinity chromatography. According to the invention the other polypeptide involves the marker his-tag. his-tag is a peptide which is constructed from 6 monomer histidine units.

A special variant of the invention is polypeptides with lipase/acyltransferase activity with an amino acid sequence which has identity to the amino acid sequence reported in SEQ ID NO 4 of at least 49%, preferably 80%, preferably at least 98%, especially preferably 99.8% and particularly 100%.

Another preferred variant of the invention is polypeptide fragments or polypeptides obtained by deletion mutation with a lipase/acyltransferase activity according to the above described polypeptides.

The term fragment refers to all proteins or peptides which are smaller than natural proteins, which are smaller than those proteins which correspond to those of SEQ ID NO 1 or SEQ ID NO 2 or SEQ ID NO 3 or SEQ ID NO 4, but are sufficiently homologous to them in the corresponding partial sequences or those which correspond to completely translated genes and, for example, can also be obtained synthetically. Based on their amino acid sequences they may be assigned to the corresponding complete proteins. For example they may assume the same structures or exercise proteolytic activities or partial activities such as for example, the complexing of a substrate. Fragments and deletion variants of initial proteins are fundamentally of the same type; while fragments rather represent smaller parts, in the case of the deletion mutants only short regions are missing and therefore only solitary partial functions.

The fragments may, for example, involve individual domains or fractions which do not agree with the domains. Such fragments may be more cheaply produced, no longer possess possibly unfavorable characteristics of the initial molecule such as possibly an activity-lowering regulation mechanism or manifest a more favorable activity profile. Such protein fragments may also be synthesized not biosynthetically but rather, for example, chemically. Chemical synthesis may be advantageous, for example, whenever chemical modifications are to be undertaken following the synthesis.

The fragments because of their fundamental similarity are also to be classified as polypeptides obtained by deletion mutation. They can agree essentially biochemically with the initial molecules or just no longer display individual functions. This appears to be especially advisable, for example, in the case of deletion of inhibiting regions. As a result the deletions may be paralleled by a specialization as well as an expansion of the field of application of the protein. To the extent that in the broadest sense lipase/acyltransferase activity is maintained, modified, specified or first achieved in this way, both the deletion variants and the fragments are proteins according to

the invention; the only additional precondition for this is that they lie within the reported similarity range to the sequences SEQ ID NO1 and SEQ ID NO 2 and SEQ ID NO 3 and SEQ ID NO4 via the still present homologous partial sequence.

Another variant of the invention is polypeptides obtained by insertion mutation or chimeric polypeptides with lipase/acyltransferase activity, which consist at least in one part of a polypeptide which is identical to an above-described polypeptide or fragment.

The term chimeric or hybrid polypeptide in the sense of the present invention refers to those proteins which are composed of elements which stem naturally from different polypeptide chains from the same organism or from different organisms. This procedure is also called shuffling or fusion mutagenesis. The sense of such a fusion may, for example, consist in bringing about or modifying a certain enzymatic function with the aid of the fusioned-on protein component. In this case in the sense of the present invention it is of no consequence whether such a chimeric protein consists of an individual polypeptide chain or several subunits among which different functions may be distributed. To realize the last-mentioned alternative, for example, it is possible post-translationally or only after a purification step by a selective proteolytic splitting to decompose a single chimeric polypeptide chain into several. The subject of the invention also includes such chimeric proteins which, on the basis of their construction via their total amino acid and/or nucleotide sequence display possibly less identity than is defined for the similarity range according to the invention but can be assigned to it in at least one of the regions introduced by fusion and in this part exercise the same functions as in a lipase/acyltransferase which falls within the above mentioned homology range over its entire length.

The term 'polypeptide obtained by insertion mutation' refers to those which were obtained by known methods by inserting a nucleic acid or protein fragment into the initial sequences. Their fundamental similarity is due to the chimeric proteins. They differ from them only in the size ratio of the unchanged protein part to the size of the entire protein. In such insertion-mutated proteins the content of foreign protein is smaller than in chimeric proteins.

Inversion mutagenesis, therefore a partial sequence reversal, can be regarded as a special form not only of deletion but also of insertion. The same is true for a new grouping of different molecule parts deviating from the original amino acid sequence. It can be regarded not only as a deletion variant, an insertion variant but also as a shuffling variant of the original protein.

Another variant of invention involves derivatives of a polypeptide with lipase/acyltransferase activity according to one of the above-described polypeptides.

In the sense of the present invention 'derivatives' means those polypeptides whose pure amino acid chain has been chemically modified. Such derivatizations may take place, for example, biologically in connection with protein biosynthesis by the host organism. Molecular biological methods may be used for this. However, they may also be conducted chemically, perhaps by chemical transformation of a side chain of an amino acid or by covalent bonding of another compound to the protein. Such a compound, for example, may involve other proteins, which, for example, are bound via bifunctional chemical compounds to the polypeptide according to the invention. The term 'derivatization' also refers to covalent binding to a macromolecular carrier. Such modifications may, for example, influence the substrate specificity or the bond strength to the substrate or bring about a temporary blocking of the enzymatic activity if the coupled-on substance is an inhibitor. This may be advisable, for example, for the time period of storage. Another variant is therefore those derivatives which are obtained by covalent binding to a macromolecular carrier such as polyethylene glycol or a polysaccharide.

In the sense of the present invention all polypeptides, enzymes, proteins, fragments and derivatives if they have not been explicitly claimed as such are summarized under the heading of polypeptides.

The enzyme activity can be modified qualitatively or quantitatively by other regions of the polypeptide which are not involved in the actual reaction itself. This involves, for example, the enzyme stability, the activity, the reaction conditions or the substrate specificity. Because, on the one hand, it is not precisely known what amino acid radicals of the polypeptide according to the invention actually catalyze hydrolysis, transesterification and esterification, and on the other hand,

certain individual functions cannot be definitively exempted in advance from involvement in catalysis. The accessory functions or partial activities include, for example, the binding of a substrate, an intermediate or final product, the activation or inhibition or mediation of a regulating effect on the hydrolytic activity. In this case, for example, the formation of a structural element may also be involved which lies far from the active center or a single peptide whose function concerns the secretion of the foreign protein out of the cell and/or its correct folding and without which in vivo as a rule no functional enzyme is formed. to be sure, hydrolysis, transesterification and esterification must all be catalyzed.

Another solution to the problem according to the invention is polypeptides or derivatives which have in common at least one antigen determinant with one of the above-mentioned polypeptides or derivatives.

Because not only is the pure amino acid sequence of a protein decisive for the exercise of enzymatic activity but also its secondary structural element and its three-dimensional folding. Thus domains deviating clearly from one another in their primary structure in three dimensions may form essentially concordant structures and thus make the same enzymatic behavior possible. Such common features in secondary structure are usually recognized as concordant antigenic determinants of antisera or pure or monoclonal antibodies. Thus similarly structured proteins or derivatives can be detected and classified by immunochemical cross reactions.

Therefore within the scope of protection of the present invention precisely those polypeptides or derivatives are included also which display lipase/acyltransferase activity and can be assigned to the above-defined proteins or derivatives according to the invention not so much because of their homology values in the primary structure but rather because of their immunochemical relationships.

Polypeptides according to the invention which stem from natural sources are preferred variants of the present invention, especially if they come from microorganisms such as single celled fungi or bacteria, because the latter can usually be handled much more simply than multicellular

organisms or cell cultures derived from multicellulars. These represent ideal options for special variants.

Especially preferred are polypeptides or derivatives according to the invention from eukaryotic fungi, especially these which can release the secreted proteins directly into the surrounding medium.

Quite especially preferred are polypeptides or derivatives according to the invention which can be obtained from microorganisms selected from the group formed by *Candida parapsilosis*, and preferably *Candida parapsilosis* CBS 604, *Candida antarctica (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathii, Issatchenkia orientalis (Candida krusei), Kluyveromyces marxianus (C. kefyr, C. pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila.*

Among the polypeptides or derivatives from Candida species according to the invention again those from *Candida parapsilosis* are preferred, including especially from *Candida parapsilosis* CBS 604, because from the latter the variant of the enzyme according to the invention whose pertinent sequences are reported in the sequence protocol was originally obtained.

For production engineering reasons, in each case, those strains are preferred which release the formed polypeptide into the medium surrounding them.

Another object of the invention is nucleic acids which code for polypeptides with lipase/acyltransferase activity whose nucleotide sequence is 100% identical to the nucleotide sequence reported in SEQ ID NO 1, especially over the partial region which corresponds to amino acids 190 through 390 according to SEQ ID NO 2. In addition nucleic acids are claimed which code for an amino acid sequence which possesses identity of at least 49% with the amino acid sequence reported in SEQ ID NO 2 preferably 80%, especially 99.8% and particularly 100%. Preferred are nucleic acids coding for an amino acid sequence which are at least 96% identical to the amino acid sequence reported in SEQ ID NO 2 in positions 190 through 390. Especially preferred are nucleic acids which code for one of the above described polypeptides or derivatives.

The similarity region also includes all polypeptides whose nucleotide sequence is at least 85%, 87.5%, 90%, 92.5%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence reported in SEQ ID NO 1.

Another object of the invention is nucleic acids which code for a polypeptide with lipase/acyltransferase activity whose nucleotide sequence is identical to the nucleotide sequence reported in SEQ ID NO 3.

Also included in the protective scope are nucleic acids coding for an amino acid sequence which is at least 49% identical preferably 80% identical to the amino acid sequence reported in SEQ ID NO 4. The similarity range also includes all polypeptides whose nucleotide sequence is identical to the nucleotide sequence reported in SEQ ID NO 3 to at least 85%, 87.5%, 90%, 92.5%, 95%, 96%, 97%, 98%, 99%, or 100%.

The term "nucleic acid" in the sense of the present invention refers to the molecules serving as information carriers constructed by natural means from nucleotides which code for the linear amino acid sequence in proteins or enzymes. They may be present as a single strand, as a single strand complementary to said single strand or as a double strand. As the naturally more long-lasting information carrier the nucleic acid DNA is preferred for molecular biological studies. As opposed to this for the realization of the invention in a natural environment such as in an expressing cell an RNA is formed, for which reason RNA molecules essential for the invention also represent variants of the present invention.

In the case of DNA the sequences of the two complementary strands are to be considered in each case in all three possible reading grids. It is also to be kept in mind that different codon triplets may code for the same amino acids so that a specific amino acid sequence may be derived from several different possibly only slightly identical nucleotide sequences (degenerateness of the genetic code). In addition different organisms display differences in the use of these codons. For these reasons both amino acid sequences as well as nucleotide sequences must be included in the scope of protection, and the reported nucleotide sequences in each case are to be regarded only as examples of coding for a specific amino acid sequence.

The information unit corresponding to a protein is also designated as a gene in the sense of the present invention.

Today for one of ordinary skill in the art who is familiar with methods such as chemical synthesis or the polymerase chain reaction (PCR) in combination with molecular biological and/or protein-chemical standard methods it is possible by using known DNA and/or amino acid sequences to synthesize the corresponding nucleic acids up to the point of complete genes. Such methods are known, for example, from "Lexikon der Biochemie", Spektrum Akademischer Verlag, Berlin, 1999, Vol 1, p 267-271 and Vol 2, p 227-229.

Changes in the nucleotide sequence such as may be caused for example, by known molecular-biological methods, are called mutations. Depending on the type of the change, one recognizes, for example, deletion, insertion or substitution mutations or those in which different genes or parts of genes are fused to each other (shuffling) ; these are gene mutations. The corresponding organisms are called mutants. The protease derived from mutated nucleic acids are designated as variants. Thus, for example, deletion, insertion, substitution mutations or fusions lead to deletion, insertion, or substitution-mutated or fusion genes and on the protein level to the corresponding deletion, insertion or substitution variants or fusion proteins respectively.

Another solution to the problem according to the invention and therefore an independent subject of the invention is organisms which naturally form a protein or derivative according to the invention or contain nucleic acids which code for a polypeptide or derivative according to the invention, because their discovery makes it possible to implement the idea of the invention. Such organisms are obtainable by application of generally well-known techniques, for example, by isolation of strains from a natural habitat, or by screening of gene banks. The nucleotide sequence reported in SEQ ID NO 1 in this case may be used, for example, as a probe for screening, or as an original for construction of corresponding PCR primers. By analogy with this short-chained or complete peptides with amino acid sequences according to SEQ ID NO 2 may be used for the formation of the corresponding antisera with the aid of which the corresponding organisms or the proteins liberated by them can be identified.

Corresponding to the statements made above are microorganisms, because the latter are established, above all, on the basis of the cultivability and as production organisms with an especially high production capacity in industrial processes, preferably yeast-fungi, including those of the genus *Candida*; *Candida parapsilosis* and quite especially *Candida parapsilosis* CBS 604 are preferred.

For production-engineering reasons, in each case, those strains are preferred which release the formed polypeptide into the medium surrounding them.

It is possible that naturally occurring producers may indeed produce an enzyme according to the invention but under the initially defined conditions express it only to a slight degree and/or release it into the surrounding medium only to a minor extent. Nevertheless they fall within the scope of the present invention as long as the possibility exist of finding experimentally suitable environmental conditions or low-molecular or other factors experimentally under whose influence they can be stimulated to produce the protein according to the invention, which appears economically favorable. Such a regulation mechanism may be used selectively for biotechnological production, e.g., for regulation of the responsible promotors.

Depending on the recovery, refining, or preparation of a protein it may be associated with various other substances, especially if it is obtained from natural producers of said protein. It may then, but also independently, be mixed selectively with certain other substances, e.g., to increase its storage stability. Therefore, the concept of the protein according to the invention also includes additionally all preparations of the actual protein essential for the invention. This is also independent of whether or not it actually manifests this enzymatic activity in a certain preparation, because it may be desirable for it to have no or only low activity during storage and manifest its function only at the time of application. This may, for example, be dependent on the state of folding of the protein or result from the reversible binding of one or more accompanying substances of the preparation or from another control mechanism.

Nucleic acid form the starting point for molecular biological studies and further developments. Such methods are described, for instance, in the manual by Fritsch, Sambrook and

Maniatis, "Molecular cloning: a laboratory manual," Cold Spring Harbour Laboratory Press, NY, 1989. All gene-technical and protein-technical methods known to the state of the art under the heading of protein engineering are based on the gene, especially the cloned gene. With them polypeptides according to the invention can be further optimized with respect to various application, e.g., by point mutagenesis or by fusion with sequences from other genes.

Vectors which contain one of the nucleic acid regions described which code for a polypeptide with lipase/acyltransferase activity according to the invention are considered to be an independent object of the invention.

In order to deal with nucleic acids the DNA is advisably cloned in a vector. Vectors are DNA molecules which are suitable transporting molecules (vehicles) for sluicing (transformation) foreign DNA into host cells and there possibly becoming autonomously replicable. Frequently used vectors are plasmids, i.e. extrachromosomal, ring-shaped, double-stranded bacterial DNA which can be introduced by suitable methods into other microorganisms and reproduced there.

The vectors include, e.g., those derived from bacterial plasmids, from viruses or bacteriophages, or predominantly synthetic vectors or plasmids with elements of a variety of origins. With the other genetic elements present in each case vectors are capable of establishing themselves in the corresponding host cells over several generations as stable units. In this case in the sense of the invention it is of no import whether they establish themselves extrachromosomally as independent units or are integrated in a chromosome. Which of the numerous systems known from the state of the art is selected depends on the individual case. For instance, the achievable number of copies, the selection systems available, including, above all, antibiotic resistances or the cultivability of the host cells capable of accepting the vectors, are of decisive importance.

The vectors form suitable starting points for molecular-biological and biochemical studies of the gene in question or of the corresponding protein and for further developments according to the invention and ultimately for amplification and production of proteins according to the invention. They represent forms of embodiment of the present invention to the extent that the sequences of the

nucleic acid regions contained according to the invention lie inside the homology range elaborated in more detail above.

Preferred embodiment forms of the present invention are cloning vectors. These are suitable, besides for storage, for biological amplification or for selection of the gene of interest, for characterization of the corresponding gene, say by preparation of a restriction map or sequencing. Cloning vectors are also preferred embodiments of the present invention, because they represent a portable and storable form of the claimed DNA. They are also preferred starting points for molecular biological techniques which are not bound to cells such as the polymerase chain reaction, for example.

Expression vectors possess partial sequences which are capable of replicating in host organisms optimized for production of proteins and of bringing the gene contained there to expression. Preferred embodiment forms are expression vectors which themselves carry the genetic elements necessary for expression. Expression, for example, is influenced by promotors which regulate the transcription of the gene. Thus the expression may take placed via the natural promotor localized originally in front of this gene but also after genetic-engineered fusion, both via a promotor of the host cell prepared on the expression vector and also via a modified or totally different promotor of another organism.

Preferred embodiment forms are those expression vectors which are capable of being regulated by changes in the culture conditions or by addition of certain compounds, e.g., the cell density or special factors. Expression vectors make it possible for the corresponding protein to be produced heterologously, therefore in a different organism than that from which it can be obtained naturally. Also a homologous protein acquisition from a host organisms expressing the gene naturally via a suitable vector lies within the scope of protection of the present invention. This may have the advantage that natural modification reactions related to the translation can be performed precisely on the protein which forms in the same manner as they would take place naturally.

To recover the polypeptide according to the invention microorganisms transformed by an expression vector containing structures coding for the corresponding enzyme are cultivated. The

expression vectors in this case were obtained by processes to be described later. The especially preferred microorganisms that were transformed with the expression vector are: *Saccharomyces cerevisiae* and *Pichi pastoris*. Preferred vectors are plasmids whose restriction maps are shown in Figures 1-3.

Among the vectors used within the scope of the invention are those which are formed by cutting with suitable restriction endonucleases, preferably BamHI or SnaBI and subsequent recombination with the corresponding N or C terminal halves of the enzyme structure gene. Restriction endonucleases are enzymes which substrate-specifically decompose double-strand DNA into fragments by splitting the phosphate diester bonds between the individual nucleotide building blocks of the DNA. All restriction endonucleases are capable of recognizing certain base sequences of the DNA which mark specific action sites (interfaces) for the activity of the corresponding endonucleases. Upon cutting (restriction) of double-strand DNA in the case of some endonucleases, specific so-called "protruding ends" are formed which, under certain conditions of renaturation, are capable of joining with each other again (recombination) or with the corresponding (complementary) protruding ends of DNA fragments (ligated) obtained by a different route.

Cell-free expression systems in which the protein biosynthesis takes place *in vitro* may also be forms of embodiment of the present invention. Such expression systems are also state of the art.

Another form of embodiment of the invented object is cells containing one of the above-defined vectors, especially a cloning or an expression vector. Their transformation into corresponding cells takes place in the course of molecular-biological operations such as are necessary, e.g., for mutagenesis, sequencing or storage of the vectors. Depending on the method, for example, here gram-positive, but especially also gram-negative bacteria may be suitable for this.

Another form of embodiment involves host cells which express a polypeptide or derivative of the primary object of the invention or can be stimulated to express them, preferably utilizing one of the above-defined expression vectors.

The preferred *in-vivo* synthesis of a polypeptide according to the invention requires the transfer of the corresponding gene to a host cell. As host cells basically all organisms are suitable,

i.e. prokaryotes, eukaryotes, or cyanophyta. Those host cells are preferred which can be readily manipulated genetically, for example, by transformation with the expression vector and its stable establishment, e.g., single-cell fungi or bacteria. Preferred host cells are characterized by good microbiological and biotechnological manipulability. This means easily cultured, high growth rates, low fermentation media requirements, and good rates of production and secretion of foreign proteins. Frequently also the plethora of different systems available as state of the art the optimal expression systems must be determined experimentally for the individual case. Every protein according to the invention can be obtained in this way from a large number of host organism.

Preferred variants are those host cells which, because of genetic regulating elements made available, e.g., on the expression vector but also already present *a priori* in these cells, are capable of being regulated in their activity; for example, they may be stimulated to expression by controlled addition of chemical compounds, such as methanol, which serve as activators, by a change in the conditions of cultivation, or upon reaching a certain cell density. This makes a very economical production of the proteins of interest possible.

A variant of this test principle is represented by expression systems in which additional genes, e.g., those made available on other vectors, influence the production of proteins according to the invention. In this case modified gene products may be involved or those that are to be purified together with the protein according to the invention, perhaps to influence its function. This may involve, e.g.,, other proteins or enzymes, inhibitors or elements influencing interaction with different substrates. Preferred host cells are prokaryote or bacterial cells. Bacteria are distinguished from eukaryotes, as a rule, by shorter generation times and lower demands on culture conditions. As a result economical processes may be set up for obtaining the proteins according to the invention.

Especially preferred are host cells, especially bacteria, which secrete the protein or derivative formed into the surrounding medium so that the expressed proteins according to the invention can be purified directly.

Heterologous expression is preferred. Gram-positive bacteria such as actinomycetes or bacilli have no external membrane so that they release the secreted proteins directly into the medium surrounding them. Among the bacteria preferred for heterologous expression are therefore those of the genus Bacillus, especially those of the species listed below.

Gram-negative bacterial may also be used for heterologous expression. Among them a large number of proteins are secreted into the periplasmatic space, i.e. the compartment between the two membranes enclosing the cells. This may be advantageous for special applications. Here belong, for instance, those of the genera *Klebsiella* or *Escherichia*, preferably of the species which are also listed below.

Eukaryote cells may also be suitable for the production of polypeptides according to the invention. Examples of these are yeasts, such as *Saccharomyces* or *Kluyveromyces*. This may be especially advantageous, for example, when the proteins are to undergo modifications in connection with their synthesis to make such systems possible. These included, for example, the binding of low molecular-weight compounds as membrane anchors or oligosaccharides.

Especially preferred for production of polypeptides according to the invention from transformed host cells are microorganisms which are selected from the group formed by *Candida parapsilosis, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Pichia boidinii, Pichia stipitis, Hansenula polymorpha, Kluyveromyces lactis, Schwanniomyces castellii, Yarrowia lipolytica, Escherichia coli, Bacillus subtilis, Bacillus amylolichefaciens, Bacillus stearothermophilus, Bacillus licheniformis, Lactococcus lactis, Streptococcus lactis, Lactobacillus bulgaricus, Aspergillus oryzae, Aspergillus niger, Trichoderma reesei, Mucor sp.* and *Rhizopus sp.*

The transformed host cells, also called transformants, are subsequently cultured by known methods, preferably as in the examples, and the formed polypeptides according to the invention are isolated.

All of the above listed elements may be combined into a process to produce polypeptides according to the invention. These processes therefore represent another object of the invention. In this case, for each protein according to the invention, a large number of possible combinations of the

process steps are conceivable. They all realize the idea on which the present invention is based, i.e. to produce quantitatively representatives of a protein type defined via the lipase/acyltransferase activity with simultaneously high homology to the sequences reported in the sequence protocols, with the aid of the corresponding genetic information. The optimal process must be determined experimentally for each specific individual case.

Fundamentally, in this case, one proceeds as follows: nucleic acids according to the invention, therefore those lying within the above-defined similarity range to the sequence of SEQ ID NO 1 or SEQ ID NO 3 are suitably ligated in a suitable expression vector in the form of DNA. The latter is transferred into the host cell, e.g., into cells of an easily cultured bacterial strain which secretes the proteins, whose genes are under the control of the corresponding genetic elements, into the surrounding nutrient medium; regulating elements for this may be made available, e.g., from the expression vector. From the surrounding medium the protein according to the invention can be isolated by several purification steps such as precipitation or chromatography. One of ordinary skill in the art is able to upscale a system that has been optimized experimentally on the laboratory scale to the scale of industrial production.

Another subject of the invention is the application of natural and/or recombinant microorganisms. such as are described above, which contain a nucleic acid, for the production of an above-described polypeptide according to the invention.

Another application according to the invention of the above-described nucleic acids and/or amino acid sequence which have identity to the amino acid sequence reported in SEQ ID NO 2 and/or to that reported in SEQ ID NO 4 of at least 49%, preferably 80%, more preferably at least 98%, especially preferably 99.8% and particularly 100% is for discovering new acyltransferases.

The discovery of new enzymes is also called screening. In particular one screens the gene banks of certain organism following general methods, such as are reported in Fritsch, Sambrook and Maniatis: "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, NY, 1989.

By comparison with known enzymes which are on file, e.g., in generally available data banks, from the amino acid or nucleotide sequence characteristic molecular parts, such as structural elements, or the enzymatic activity of an enzyme in question may be inferred. Such a comparison is accomplished by coordinating similar sequences in the nucleotide or amino acid sequences of the proteins being analyzed with each other. This is called "homologizing." A tabular classification of the positions involved is called an "alignment." In the analysis of nucleotide sequences, in turn, both complementary strands of all three possible reading grids are to be taken into account. Likewise, the degenerateness of the genetic code and the organism-specific codon usage. In the meanwhile alignments are prepared by computer program, such as the algorithms FASTA or BLAST; this procedure is described, e.g., by D. J. Lipman and W. R. Pearson (1985) in Science, vol. 227, p 1435-1441. A comparison of all positions in agreement in the sequences compared is called "consensus sequence."

Such a comparison also permits a pronouncement to be made regarding the similarity or homology of the sequences being compared with each other. This is reported in "per cent identity," i.e. the content of identical nucleotides or amino acid radicals in the same positions. Another homology definition relates the preserved amino acid exchanges in this value to unity. One then speaks of "per cent similarity." Such statements may be made about entire proteins or genes or only about individual regions.

Homologous regions of different proteins are usually those with the same structural elements and/or functions which may be recognized by agreements in the primary amino acid sequence. It ranges up to full identities in the smallest regions, so-called boxes, which include only a few amino acids and usually exercise essential functions for the overall activity. The term "functions of the homologous regions" are to be understood to mean the smallest partial functions of the function exercised by the total protein, such as, e.g., the formation of individual hydrogen bridge bonds for complexing a substrate or transition complex.

Based on alignments, essentially the same secondary and tertiary structures may be assumed for polypeptides according to the invention as for the proteins used for homologization. Their

structural elements can generally be called up in the generally accessible data banks, such as that at the EMBL-European Bioinformatics Institute (EBI) in Cambridge, Great Britain (http://www.ebi.ac.uk), Swiss-Prot. or GenBank (National Center for Biotechnology Information, NCB, National Institutes of Health, Bethesda, USA.). If structures deviating from them should arise or if it should be found that different folding variants exist with varying properties, which concerns, e.g., the optimal reaction conditions or substrate specificity, then all of these are included within the scope of protection of the present invention. Because, on the one hand, the folding may depend on the production conditions, e.g., in the presence or absence of the leader peptide. On the other hand, these variants may proved especially suitable for different possible uses in each case.

Another subject of the invention is the application of polypeptides described as catalysts in acyl transfer reactions, especially in reactions selected from the group formed by alcoholysis of esters, especially glycerols or sterols, alcoholysis of thio esters, thiolysis of esters, aminolysis of an ester with hydroxylamines or hydrazines; reaction of an ester with hydrogen peroxides and enantioselective synthesis of esters, thioesters or lactones by alcoholysis. Special reactions which are catalyzed by the polypeptides according to the invention are described, e.g., in a) Fournand, et al., J. Mol. Catalysis B, 1998, 5, 207-211; b) Briand, et al., Eur. J. Biochem. 1995, 228, 169-75.

Examples

Example 1. Cultivation of the strain and isolation of the polypeptide

The strain *Candida parapsilosis* (Ashford) Langeron and Tallice, CBS 604, was filed at the Centraalbureau voor Schimmelcultures, Yeast Division, Delft, Netherlands.

The cultivation was carried out in the same manner as in Briand, et al., Eur. J. Biochem. 1995, 228, 169-175. The main culture was adjusted to pH 6.5 with 100 mM phosphate buffer and mixed with 5 g/l glucose as the C source.

At the end of the exponential growth phase the culture broth was centrifuged (7000 g for 15 min) and the lipase/acyltransferase obtained from the supernatant fluid. The polypeptide was purified by the method described in Riaublanc, A., et al., J. Am. Oil Chem. Soc. 1993, 70, 497-500.

Example 2. Molecular-biological operating steps

All molecular-biologic operating steps follow standard methods such as are reported in the manuals such as that of Fritsch, Sambrook, and Maniatis, "Molecular Cloning: A laboratory manual," Cold Spring Harbor Laboratory Press, NY, 1989.

The content of lipase/acyltransferase was measured in units (U) determined as the content of oleic acid which was obtained per minute during the hydrolysis of trioleyl glycerol under the conditions described in Briand et al., in Eur. J. Biochem. 1995, 228, 169-175. The protein concentration was determined by the method of Bradford (1976, Anal. Biochem. 72, 248-254).

Example 3: Expression of a gene containing the nucleic acid per SEQ ID NO 1 in *Saccharomyces cerevisiae*

For expression of the desired nucleic acid sequence the DNA was partially hydrolyzed with the restriction endonuclease BamHI. Using degenerated PCR primers were constructed which contained the nucleic acid per SEQ ID NO 1. The following primer pairs were used (start and stop codon are underlined, BamHI restriction side is printed in bold face type):

forward 5'-CTCGGATCCATGCGTTACTTTGCTATTGC
reverse 5'-CACGGATCCTTAAAAAGCAAAACGTTCCAACTTGAGCAATCC

The following time/temperature program was carried out for PCR amplification: 5 min at 95°C denaturing and then 30 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C and as the last step 10 min at 72°C.

The fragments from the PCR were digested with the restriction endonuclease BamHI and subsequently ligated into the vector pVT100-U cut with the restriction endonuclease BamHI to form the plasmid. The vector pVT100CpLIP2 shown in Figure 1 (replicative plasmid) was obtained. The absence of mutations was verified by sequencing the insert. The transformation of the newly combined DNA into the strain *Saccharomyces cerevisiae* W303-1a was performed by the

electroporation method described by Becker et al. in Methods in Enzymology, 1991, 194, 182-187. The transformants were selected on YNB medium without uracil (6.7 g/l Yeast Nitrogen Base without amino acid by Difco, 20 g/l glucose, 150 mg/l leucine, 100 mg/l adenine, 100 mg/l histidine, 100 mg/l tryptophan) with a frequency of 1-2x10^4 transformants per µg of DNA. The transformants were selected in a plate test for lipase activity by the method of Kouker described in: Kouker, G., et al., Applied Environ. Microbiol. 1987, 59, 211-213.

The transformant selected was cultured in a shaker bottle at 28°C in YPD medium (YPD=10 g/l yeast extract by Difco, 20 g/l Bacto Peptone [Difco], 60 g/l glucose, 150 mg/l leucine, 100 mg/l adenine, 100 mg/l histidine and 100 mg/l tryptophan). The culture broth was harvested after 36 h of fermentation, and the supernatant of the culture solution was separated from the residue by centrifugation. The supernatant contained 2500 U of the recombinant lipase/acyltransferase per liter and a specific activity of 0.7 U/mg. After concentrating by ultrafiltration and hydrophobic chromatography on phenylsepharose 6 Fast-Flow gel 10% of the activity could be recovered with a specific activity of 80 U/mg.

Example 4: Expression of a gene containing SEQ ID NO 1 in *Pichia pastoris*

The lipase/acyltransferase was expressed as fusion to an N terminal peptide which coded for the secretion signal of the α factor from *Saccharomyces cerevisiae.* First the gene corresponding to SEQ ID NO 1 PCR was amplified and in this way a cut gene of the mature gene was obtained. The following primers were used: (the stop codon is underlined, the first phenylalanine codon of the mature gene is printed in boldface type):

forward 5'-TTTGTCTTGGCTCCCAAAAAGCCA
reverse 5'-TTAAAAAGCAAAACGTTCCAACTTGAGCAATCC

The following time/temperature program was executed for PCR amplification: 2 min at 94°C denaturing and then 15 cycles of 15 sec at 94°C, 30 sec at 50°C, 90 sec at 72°C plus 5 sec per cycle for the extension period of cycle 11, and as the last step, 7 min at 72°C.

After amplification the fragment obtained was phosphorylated with T4 polynucleotide lipase and blunted off with T4-DNA polymerase. The fragment was then ligated with a pPIC9K plasmid digested by SnaBI, and the vector pPIC9KCpLIP2 (replicative plasmid) was obtained (Figure 2). The absence of mutations was verified by sequencing the insert.

The transformation of the yeast spheroplasts was performed with the Pichia expression kit by the Invitrogen Co. (Groningen, Netherlands). The transformation frequency was 10^3 transformations per µg of DNA.

A selected transformant was cultivated in a fermenter with a synthetic described by Boze et al. in: Boze, H., et al., Process Biochem. 2001, 36, 907-913) to which 40 g/l glycerol had been added. After the growth phase (after 2500 min. of fermentation) in the batch process pure methanol (5 g/l) was added in the sub-batch process in order to induce the expression of the gene. After four days of cultivation with a high cell density the supernatant of the culture broth is separated from the residue by centrifugation. The supernatant obtained contained 102000 U/l of recombinant lipase\acyltransferase with a specific activity of 80 U/mg of protein. Concentration by ultrafiltration with 10000 kD cut-off membranes produced an enzyme concentration of 830000 U/l with a specific activity of 150 U/mg.

Example 5: Expression of a modified (His-tagged) lipase/acyltransferase in *Saccharomyces cerevisiae*

In order to express the modified desired nucleic acid sequence per SEQ ID NO 3 which makes possible the fusion of 6-His-peptide to the C-terminal end of the sequence of the polypeptide per SEQ ID NO 2 first DNA was partially hydrolyzed with the restriction endonuclease BamHI. Primers were constructed by PCR which contained the nucleic acid per SEQ ID NO 1. The following primer pairs, which are made possible by an extension of the nucleic acid sequence with 6 histidine codons, were used (start and stop codon are underlined, BamHI restriction side printed in boldface type, his codons are printed in cursive):

forward 5'-CTCGGATCCATGCGTTACTTTGCTATTGC
reverse 5'-CACGGATCCTTAATGATGATGATGATGATGAAAAGCAAAACGTTCCAACTTGAGCAATCC

The following time/temperature program was carried out for PCR amplification: denaturing for 5 min at 95°C and then 30 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C, and as the last step 10 min at 72°C.

The fragments from PCR were digested with the restriction endonuclease BamHI and subsequently ligated to the plasmid in the vector pVT100-U cut with the restriction endonuclease BamHI. The vector pVT100CpLIP2His in Figure 3 (integrative plasmid) was obtained. The transformation of *Saccharomyces cerevisiae* W303-1a and the expression of the gene were carried out according to example 3.

The transformant selected was cultivated in shaker bottles at 28°C in YPD medium (YPD = 10 g/l yeast extract [Difco], 20 g/l Bacto Peptone [Difco], 60 g/l glucose, 150 mg/l leucine, 100 mg/l adenine, 100 mg/l histidine, and 100 mg/l tryptophan). The culture broth was harvested after 36 hours of fermentation, and the supernatant of the culture solution was separated from the residue by centrifugation. The supernatant contained 3100 U of the recombinant his-tagged lipase/acyltransferase per liter and had as specific activity of 0.25 U/mg of protein. Ion-chelating properties were utilized for purification in one step. For this purpose Ni-nitrilotriacetic acid agarose affinity gel by the Qiagen Co. were used as described by the manufacturer. 26% of the enzyme was obtained with a specific activity 150 U/mg of protein.

SEQUENCE LISTING

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<140>
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<160> 4

<170> PatentIn Ver. 2.1

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96
Phe Val Leu Ala Pro Lys Lys Pro Ser Gln Asp Asp Phe Tyr Thr Pro
20 25 30

cca caa ggt tat gaa gct caa cct ctt ggt tct att ttg aaa aca aga
144
Pro Gln Gly Tyr Glu Ala Gln Pro Leu Gly Ser Ile Leu Lys Thr Arg
35 40 45

aac gtc ccc aat cca ttg act aat gtt ttc act cca gtt aaa gtt caa
192
Asn Val Pro Asn Pro Leu Thr Asn Val Phe Thr Pro Val Lys Val Gln
50 55 60

aat gca tgg caa tta ttg gtt aga tct gaa gat aca ttt ggt aac cca
240

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aac gcc ata gtc act acc att att caa cct ttc aat gct aaa aag gat
288
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aag ctt gtt tct tat caa aca ttt gaa gat tct ggt aaa ttg gat tgt
336
Lys Leu Val Ser Tyr Gln Thr Phe Glu Asp Ser Gly Lys Leu Asp Cys
            100                 105                 110

gct cca tca tat gct att caa tat gga tcg gac att tcg act ttg acc
384
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432
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480
Tyr Val Val Thr Pro Asp Tyr Glu Gly Pro Lys Ser Thr Phe Thr Val
145                 150                 155                 160

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576
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624
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caa aaa gaa tat gct cca gag ttg agt aaa aac ttg ctt ggt gct gca
672
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768
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816
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864
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960
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1008
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1056
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1104
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1344
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Phe
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 65                  70                  75                  80
Asn Ala Ile Val Thr Thr Ile Ile Gln Pro Phe Asn Ala Lys Lys Asp
                 85                  90                  95
Lys Leu Val Ser Tyr Gln Thr Phe Glu Asp Ser Gly Lys Leu Asp Cys
            100                 105                 110
Ala Pro Ser Tyr Ala Ile Gln Tyr Gly Ser Asp Ile Ser Thr Leu Thr
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96
Phe Val Leu Ala Pro Lys Lys Pro Ser Gln Asp Asp Phe Tyr Thr Pro
             20                  25                  30

cca caa ggt tat gaa gct caa cct ctt ggt tct att ttg aaa aca aga
144
Pro Gln Gly Tyr Glu Ala Gln Pro Leu Gly Ser Ile Leu Lys Thr Arg
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288
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aag ctt gtt tct tat caa aca ttt gaa gat tct ggt aaa ttg gat tgt
336
Lys Leu Val Ser Tyr Gln Thr Phe Glu Asp Ser Gly Lys Leu Asp Cys
            100                 105                 110

gct cca tca tat gct att caa tat gga tcg gac att tcg act ttg acc
384
Ala Pro Ser Tyr Ala Ile Gln Tyr Gly Ser Asp Ile Ser Thr Leu Thr
        115                 120                 125

act caa ggt gaa atg tac tac atc tct gct tta tta gat caa ggt tac
432
Thr Gln Gly Glu Met Tyr Tyr Ile Ser Ala Leu Leu Asp Gln Gly Tyr
    130                 135                 140

tat gtt gtc act cct gat tac gag ggt cca aag agt aca ttc act gta
480
Tyr Val Val Thr Pro Asp Tyr Glu Gly Pro Lys Ser Thr Phe Thr Val
145                 150                 155                 160

ggg ttg caa tca gga aga gct act ttg aat tcg ctt aga gct act ttg
528
Gly Leu Gln Ser Gly Arg Ala Thr Leu Asn Ser Leu Arg Ala Thr Leu
                165                 170                 175

aaa tca gga aac ttg act ggt gtt tca tca gac gct gag aca tta ttg
576
Lys Ser Gly Asn Leu Thr Gly Val Ser Ser Asp Ala Glu Thr Leu Leu
            180                 185                 190

tgg ggt tat tca gga gga agt ctt gct tca gga tgg gct gct gct ata
624
Trp Gly Tyr Ser Gly Gly Ser Leu Ala Ser Gly Trp Ala Ala Ala Ile
        195                 200                 205

caa aaa gaa tat gct cca gag ttg agt aaa aac ttg ctt ggt gct gca
672
Gln Lys Glu Tyr Ala Pro Glu Leu Ser Lys Asn Leu Leu Gly Ala Ala
    210                 215                 220

ctt ggt gga ttc gtt aca aac att act gcc act gct gaa gct gtt gat
720
Leu Gly Gly Phe Val Thr Asn Ile Thr Ala Thr Ala Glu Ala Val Asp
225                 230                 235                 240
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agt ggt cca ttt gca gga atc atc tcc aat gca ttg gct ggt att gga
768
Ser Gly Pro Phe Ala Gly Ile Ile Ser Asn Ala Leu Ala Gly Ile Gly
                245                 250                 255

aat gaa tac cct gat ttc aaa aac tat ctt ttg aaa aaa gtg tca cca
816
Asn Glu Tyr Pro Asp Phe Lys Asn Tyr Leu Leu Lys Lys Val Ser Pro
            260                 265                 270

ttg ctt tca atc act tat cgt ttg gga aac act cac tgt ttg ctt gat
864
Leu Leu Ser Ile Thr Tyr Arg Leu Gly Asn Thr His Cys Leu Leu Asp
        275                 280                 285

ggt ggt att gct tat ttc ggt aaa tca ttc ttt tcc aga att att aga
912
Gly Gly Ile Ala Tyr Phe Gly Lys Ser Phe Phe Ser Arg Ile Ile Arg
    290                 295                 300

tat ttc cct gat gga tgg gat ctt gtc aac caa gaa cct atc aaa acc
960
Tyr Phe Pro Asp Gly Trp Asp Leu Val Asn Gln Glu Pro Ile Lys Thr
305                 310                 315                 320

atc ttg caa gat aat gga ttg gtt tac caa cca aag gac ttg acc cca
1008
Ile Leu Gln Asp Asn Gly Leu Val Tyr Gln Pro Lys Asp Leu Thr Pro
                325                 330                 335

caa att cca tta ttc atc tac cac ggt acc ttg gat gca att gtc ccc
1056
Gln Ile Pro Leu Phe Ile Tyr His Gly Thr Leu Asp Ala Ile Val Pro
            340                 345                 350

att gtc aac tca aga aag aca ttc caa caa tgg tgt gat tgg gga ctc
1104
Ile Val Asn Ser Arg Lys Thr Phe Gln Gln Trp Cys Asp Trp Gly Leu
        355                 360                 365

aaa tct ggt gaa tat aat gaa gat ttg acc aat gga cac att act gaa
1152
Lys Ser Gly Glu Tyr Asn Glu Asp Leu Thr Asn Gly His Ile Thr Glu
    370                 375                 380
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tca att gtg ggt gca cca gct gct ttg act tgg att atc aat cgt ttc
1200
Ser Ile Val Gly Ala Pro Ala Ala Leu Thr Trp Ile Ile Asn Arg Phe
385                 390                 395                 400

aat gga cag cct cca gtt gat gga tgt caa cat aat gtg aga gct tca
1248
Asn Gly Gln Pro Pro Val Asp Gly Cys Gln His Asn Val Arg Ala Ser
                405                 410                 415

aac ttg gaa tat cca gga act cca caa tca atc aag aat tac ttt gaa
1296
Asn Leu Glu Tyr Pro Gly Thr Pro Gln Ser Ile Lys Asn Tyr Phe Glu
            420                 425                 430

gct gca ttg cac gca att ttg ggc ttt gat ttg ggt cca gat gtt aag
1344
Ala Ala Leu His Ala Ile Leu Gly Phe Asp Leu Gly Pro Asp Val Lys
        435                 440                 445

aga gat aag gtt act ttg ggc gga ttg ctc aag ttg gaa cgt ttt gct
1392
Arg Asp Lys Val Thr Leu Gly Gly Leu Leu Lys Leu Glu Arg Phe Ala
    450                 455                 460

ttt cat cat cat cat cat cat taa
1416
Phe His His His His His His
465                 470
```

<210> SEQ ID NO 4

<211> LENGTH: 471
<212> TYPE: PROTEIN
<213> ORGANISM: Candida parapsilosis

<400> 4

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Met Arg Tyr Phe Ala Ile Ala Phe Leu Leu Ile Asn Thr Ile Ser Ala
  1               5                  10                  15
Phe Val Leu Ala Pro Lys Lys Pro Ser Gln Asp Asp Phe Tyr Thr Pro
             20                  25                  30
Pro Gln Gly Tyr Glu Ala Gln Pro Leu Gly Ser Ile Leu Lys Thr Arg
         35                  40                  45
Asn Val Pro Asn Pro Leu Thr Asn Val Phe Thr Pro Val Lys Val Gln
     50                  55                  60
Asn Ala Trp Gln Leu Leu Val Arg Ser Glu Asp Thr Phe Gly Asn Pro
 65                  70                  75                  80
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Asn Ala Ile Val Thr Thr Ile Ile Gln Pro Phe Asn Ala Lys Lys Asp
                 85                  90                  95
Lys Leu Val Ser Tyr Gln Thr Phe Glu Asp Ser Gly Lys Leu Asp Cys
            100                 105                 110
Ala Pro Ser Tyr Ala Ile Gln Tyr Gly Ser Asp Ile Ser Thr Leu Thr
        115                 120                 125
Thr Gln Gly Glu Met Tyr Tyr Ile Ser Ala Leu Leu Asp Gln Gly Tyr
    130                 135                 140
Tyr Val Val Thr Pro Asp Tyr Glu Gly Pro Lys Ser Thr Phe Thr Val
145                 150                 155                 160
Gly Leu Gln Ser Gly Arg Ala Thr Leu Asn Ser Leu Arg Ala Thr Leu
                165                 170                 175
Lys Ser Gly Asn Leu Thr Gly Val Ser Ser Asp Ala Glu Thr Leu Leu
            180                 185                 190
Trp Gly Tyr Ser Gly Gly Ser Leu Ala Ser Gly Trp Ala Ala Ala Ile
        195                 200                 205
Gln Lys Glu Tyr Ala Pro Glu Leu Ser Lys Asn Leu Leu Gly Ala Ala
    210                 215                 220
Leu Gly Gly Phe Val Thr Asn Ile Thr Ala Thr Ala Glu Ala Val Asp
225                 230                 235                 240
Ser Gly Pro Phe Ala Gly Ile Ile Ser Asn Ala Leu Ala Gly Ile Gly
                245                 250                 255
Asn Glu Tyr Pro Asp Phe Lys Asn Tyr Leu Leu Lys Lys Val Ser Pro
            260                 265                 270
Leu Leu Ser Ile Thr Tyr Arg Leu Gly Asn Thr His Cys Leu Leu Asp
        275                 280                 285
Gly Gly Ile Ala Tyr Phe Gly Lys Ser Phe Phe Ser Arg Ile Ile Arg
    290                 295                 300
Tyr Phe Pro Asp Gly Trp Asp Leu Val Asn Gln Glu Pro Ile Lys Thr
305                 310                 315                 320
Ile Leu Gln Asp Asn Gly Leu Val Tyr Gln Pro Lys Asp Leu Thr Pro
                325                 330                 335
Gln Ile Pro Leu Phe Ile Tyr His Gly Thr Leu Asp Ala Ile Val Pro
            340                 345                 350
Ile Val Asn Ser Arg Lys Thr Phe Gln Gln Trp Cys Asp Trp Gly Leu
        355                 360                 365
Lys Ser Gly Glu Tyr Asn Glu Asp Leu Thr Asn Gly His Ile Thr Glu
    370                 375                 380
Ser Ile Val Gly Ala Pro Ala Ala Leu Thr Trp Ile Ile Asn Arg Phe
385                 390                 395                 400
Asn Gly Gln Pro Pro Val Asp Gly Cys Gln His Asn Val Arg Ala Ser
                405                 410                 415
Asn Leu Glu Tyr Pro Gly Thr Pro Gln Ser Ile Lys Asn Tyr Phe Glu
            420                 425                 430
Ala Ala Leu His Ala Ile Leu Gly Phe Asp Leu Gly Pro Asp Val Lys
        435                 440                 445
Arg Asp Lys Val Thr Leu Gly Gly Leu Leu Lys Leu Glu Arg Phe Ala
```

450 455 460

Phe His His His His His His

465 470

What is claimed is:

1. An isolated polypeptide comprising SEQ ID NO:2.

2. Polypeptide with lipase/acyltransferase activity with an amino acid sequence which is identical to the amino acid sequence reported in SEQ ID NO 2 in positions 190 to 390 to at least 96%.

3. Polypeptide with lipase/acyltransferase activity with an amino acid sequence which is identical to the amino acid sequence reported in SEQ ID NO 2.

4. Polypeptide as in one of claims 1-3 characterized by the fact that it is present in glycosylated form.

5. Polypeptide as in claims 1-3 characterized by the fact that it is linked to another peptide.

6. Polypeptide as in claim 5 characterized by the fact that the other peptide is a marker, preferably a his-tag.

7. Polypeptide with an amino acid sequence which possesses identity to the amino acids sequence reported in SEQ ID NO 4 of at least 49%, preferably 80%.

8. Polypeptide with an amino acid sequence which is 100% identical to the amino acid sequence reported in SEQ ID NO 4.

9. Polypeptide fragments or polypeptides obtained by deletion mutation with lipase/acyltransferase activity according to one of claims 1-8.

10. Polypeptides obtainable by insertion mutation or chimeric polypeptides having lipase/acyltransferase activity which consist at least in a part of a polypeptide which is identical to a polypeptide or fragment according to one of claims 1-9.

11. Derivatives of a polypeptide with lipase/acyltransferase activity according to one of claims 1-10.

12. Polypeptide with lipase/acyltransferase activity which have at least one antigenic determinant in common with one of the polypeptides or derivatives named in claims 1-11.

13. Polypeptide as in at least one of claims 1-12 characterized by the fact that they are obtainable naturally from a microorganism.

14. Polypeptides as in claim 13 characterized by the fact that the microorganism involves a eukaryote fungus, preferably a yeast fungus.

15. Polypeptides as in claim 13 characterized by the fact that they are obtainable from microorganism selected from the group formed by *Candida parapsilosis, Candida antarctica, (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathii, Issatchenkia orientalis (Candida krusei, Kluyveromyces marxianus (C. kefyr, C. pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila.*

16. Nucleic acids coding for a polypeptide with lipase/acyltransferase activity whose nucleotide sequences are identical to the nucleotide sequence reported in SEQ ID NO 1 to 100%, especially over the partial region which corresponds to amino acids 190-390 according to SEQ ID NO 2.

17. Nucleic acids coding for an amino acid sequence which possesses identity to the amino acid sequence reported in SEQ ID NO 2 of at least 49%, preferably 80%.

18. Nucleic acids coding for an amino acid sequence which is identical to the amino acid sequence reported in SEQ ID NO 2 in positions 190-390 to at least 96%.

19. Nucleic acids coding for one of the polypeptides or derivatives designated in claims 1-15.

20. Nucleic acids coding for a polypeptide with lipase/acyltransferase activity whose nucleotide sequence is identical to the nucleotide sequence reported in SEQ ID NO 3.

21. Nucleic acids coding for an amino acid sequence which possesses identity to the amino acid sequence reported in SEQ ID NO 4 of at least 49%, preferably 80%.

22. Organism which forms the proteins or derivatives noted in claims 1-15 or contains nucleic acids coding for them.

23. Organism as in claim 22 characterized by the fact that it is a microorganism, preferably a yeast fungus.

24. Organism as in one of claims 22 and/or 23 selected from the group formed by *Candida parapsilosis, Candida antarctica (Trychosporon oryzae, Pseudozyma antarctica), Candida glabrata, Candida albicans, Candida maltosa, Candida tropicalis, Candida viswanathii, Issatchenkia orientalis, (Candida krusei), Kluyveromyces marxianus (C. kefyr, C. Pseudotropicalis), Pichia guilliermondii (Candida guilliermondii), Geotrichum candidum, Fusarium solani* and *Aeromonas aerophila.*

25. Vector containing the nucleic acid designated in claims 16-21 which codes for one of the polypeptides or derivatives denoted in claims 1-15.

26. Cloning vector according to claim 25.

27. Expression vector as in claim 25.

28. Cell which contains a vector according to one of claims 25-27.

29. Transformed host cell which expresses one of the polypeptides or derivatives designated in claims 1-15 or can be stimulated to its expression, preferably by using an expression vector according to claim 27.

30. Transformed host cell as in claim 29 containing a nucleic acid which codes for an amino acid sequence which possesses identity to the amino acid sequence reported in SEQ ID NO 2 of 100%.

31. Transformed host cell as in claims 29 and/or 30 containing a nucleic acid which codes for an amino acid sequence which possesses identity to the amino acid sequence reported SEQ ID NO 2 of at least 49%, preferably 80%.

32. Transformed host cell as in claim 29 containing a nucleic acid which codes for an amino acid sequence which has identity to the amino acid sequence reported SEQ ID NO 4 of 100%.

33. Transformed host cell as in claim 29 and/or 30 containing a nucleic acid which codes for an amino acid sequence which possesses identity to the amino acid sequence reported in SEQ ID NO 4 of at least 49%, preferably 80%.

34. Transformed host cell as in one claim 29-33 characterized by the fact that the host cells being transformed are host cells from microorganisms.

35. Transformed host cells as one of claims 29-34 characterized by the fact that the host cells undergoing transformation are host cells from microorganisms which are selected from the group formed by *Candida parapsilosis, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Pichia boidinii, Pichia stipitis, Hansenula polymorpha, Kluyveromyces lactis, Schwanniomyces castellii, Yarrowia lipolytica, Escherichia coli ,Bacillus subtilis, Bacillus amylolichefaciens, Bacillus stearothermophilus, Bacillus licheniformis, Lactococcus lactis, Streptococcus lactis, Lactobacillus bulgaricus, Aspergillus oryzae, Aspergillus niger, Trichoderma reesei, Mucor sp.* and *Rhizopus sp.*

36. Process for production of a polypeptide as in at least one of claims 1-15 by using a nucleic acid which codes for an amino acid sequence which has identity to the amino acid sequence reported in SEQ ID NO 2 of at least 49%, preferably 80%, and/or by using a vector according one of claims 25-27 and/or by using a transformed host cell according one of claims 29-35 or by using a cell which forms these naturally, especially a cell from an organism according to claims 22-24.

37. Application of natural and/or recombinant microorganisms containing a nucleic acid for production of a polypeptide according to at least one of claims 1-15.

38. Application of a nucleic acid according to claims 16-21 and/or application of amino acid sequences which possess identity to the amino acid sequence reported in SEQ ID NO 2 of at least 49%, preferably 80%, in order to discover new acyltransferases.

39. Application of a nucleic acid according to claims 16-21 and/or the use of amino acid sequences which possess identity to the amino acid sequence reported in the SEQ ID NO 4 of at least 49%, preferably 80%, in order to discover new acyltransferases.

40. Application of polypeptides as in claims 1-15 as catalysts in acyl transfer reactions, especially the reactions which are selected from the group formed by alcoholysis of esters, alcoholysis of thio esters, thiolysis of esters, aminolysis of an ester with hydroxylamines or

hydrazines, reaction of an ester with hydrogen peroxides and enantioselective synthesis of esters, thioesters, and lactones by alcoholysis.

Figure 1: Vector pVT100CpLIP2

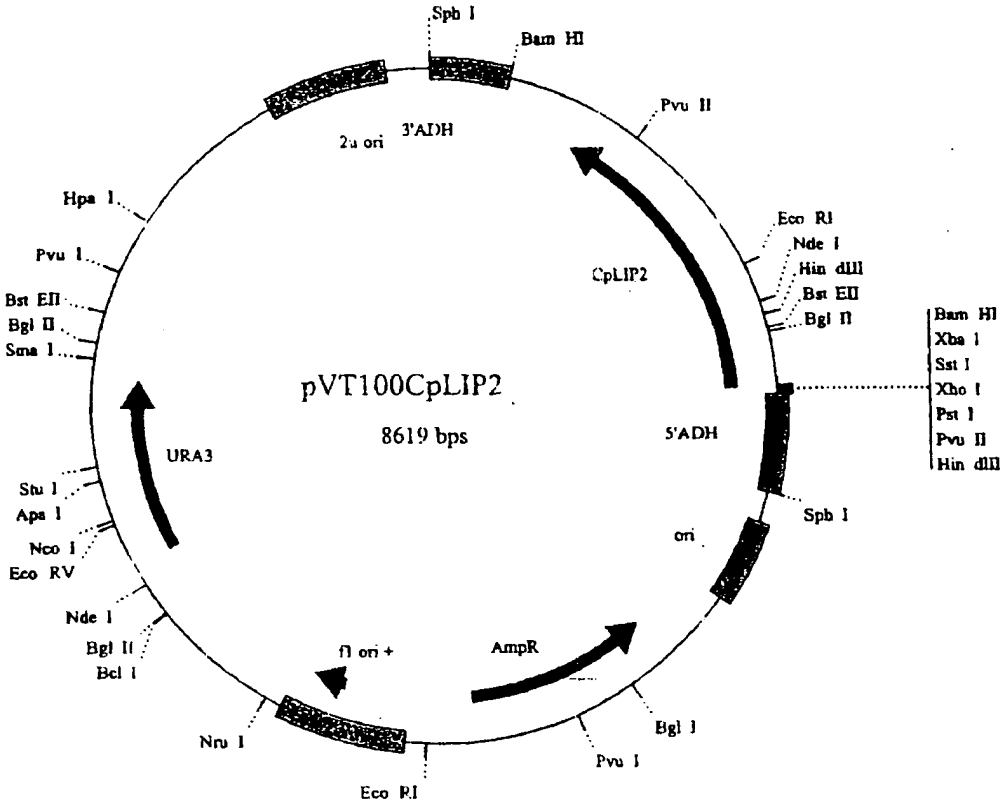

Figure 2: Vector pPIC9KCpLIP2

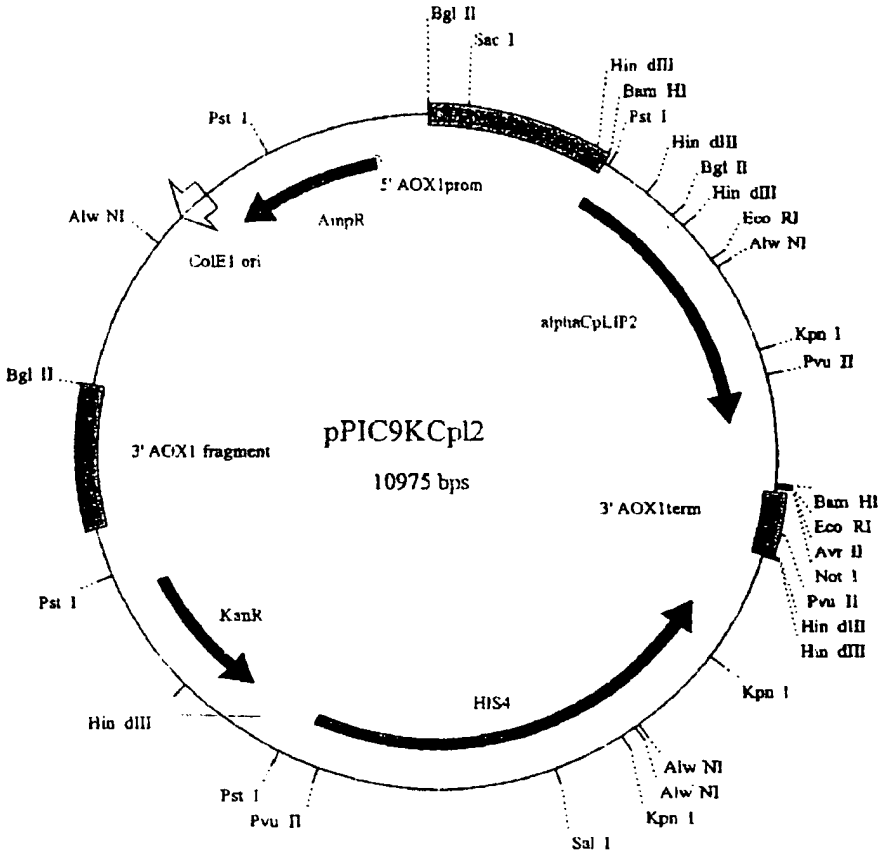

Figure 3: Vector pVT100CpL1P2His